4.—The fungus Panus fasciatus (Pleurotaceae) characterised by microstructure of sporophore and culture

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Abstract

The techniques of hyphal analysis and growth on standardised media were used to compare three collections of *Panus fasciatus*, two from Western Australia and the other from New South Wales. Although the sporophores appeared similar macroscopically and microscopically, the cultures from Western Australia differed in growth rate, texture, colour and odour, from those of New South Wales, whilst being similar in their reaction to gallic and tannic acid incorporated in the media and in certain hyphal structures. They are considered to be different varieties of the same species. Another collection from New South Wales, named *Lentinus terrestris* Lloyd, is demonstrably different even at the generic level, although synonymy with *Panus fasciatus* had been suggested.

Introduction

The taxonomy of the wood-attacking gill fungi, of which *Panus fasciatus* is an example, has been complicated by the ease with which they could be preserved as specimens by the early botanical collectors in various parts of the world. As these collectors did not realise the importance of collecting sporing specimens, and collecting them in sufficient quantity to represent developmental stages and phenotypic variation, numerous taxa have been erected on inadequate material poorly described. The object of the work reported here was to take several collections generally ascribable to *Panus fasciatus* and apply to them full micro-anatomical analysis, that might contribute to their taxonomy, supplementing this with equally exhaustive analysis of cultural characteristics. This combination of sporophore and culture analysis is seen as an essential in the elucidation of wood-attacking fungi (including the polypores) and, as this is the first time these tools have been applied to Western Australian collections, are reported in some detail. No attempt is made to make taxonomic decisions, which will depend on more extensive collections and comparisons with type specimens. However, the features that appear to be important in taxonomy are pointed out and it is shown that *Lentinus terrestris* Lloyd, considered by Cleland (1934 p 171) as probably synonymous with *Panus fasciatus*, must be a separate species on the basis of culture DFP 7396 and its corresponding sporophore.

Methods

Fresh sporophores were described macroscopically and microscopically, colour descriptions being those of Ridgway (1912). Thin sections of sporophores were mounted in 10% potassium hydroxide containing 1% aqueous phloxine to stain the trama, hymenial layer and hyphal elements. Melzer's Reagent was used to determine whether spores were amyloid or not.

Cultures were prepared from fresh sporophores and grown on 1.2% Malt Extract Agar as described by Nobles, 1965. Oxidase reactions with gallic and tannic acid were determined by Bavendamm's method as described by Davidson, Campbell and Blaisdell, 1938.

Cultures were examined microscopically after two weeks' incubation in the dark at 25°C, mounts of mycelium being from:—

(1) the advancing zone of the colony, (2) the aerial mycelium at a point one week's growth behind the margin, (3) submerged mycelium below point (2), (4) aerial mycelium at the point of two weeks' growth behind the margin, (5) submerged mycelium at the same point as (4).

Colour descriptions of hyphae and spores were made from water mounts without heat treatment. Mounts for measurements and detailed microscopic analysis were made in 10% potassium hydroxide and 1% phloxine, as used for sporophore material.

Description of Panus fasciatus (Berk.) Pegler from Western Australia

Culture WW1 was isolated from sporophores growing on decayed wood collected in Tutanning Reserve, Western Australia, August 1966 UWA. Mycology Herbarium number 1250. Specimens sent to the Royal Botanic Gardens Kew, were determined by Mr. D. N. Pegler as *P. fasciatus* (Berk.) Pegler, a fungus collected in Tasmania and described by Berkeley as *Lentinus fasciatus* (Pegler, 1965).

Culture XX1 was isolated from an identical fungus collected from a fallen dead trunk of *Eucalyptus marginata*, Karnet, Western Australia, August 1966. UWA Mycology Herbarium number 1260.

Sporophores

Sporophores tough when fresh, hard when dried. Pilei deeply infundibuliform, densely hispid with involute margins. Clay to Tawny Olive, diameter 1.2-3 cm. Gills deeply decurrent, crowded, entire along the edge, tinged pale purple when fresh but Light Mouse Gray when dried. Stipes, central, 1.0-2.5 cm, densely hispid and brown (Fig. 1).

Pileus with filamentous cuticle and white context. Dimitic: skeletal hyphae mainly in the trama (Fig. 2B) and hyaline, thick-walled, septate, clamped, and occasionally branched, with narrow lumen, 3 — 5 μ wide, mean 3 $\pm$ 0.1 μ. In contrast, generative hyphae thin-walled and,

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Figure 1.—*Panus fasciatus* from Western Australia. Sporophores corresponding to culture number XX1.

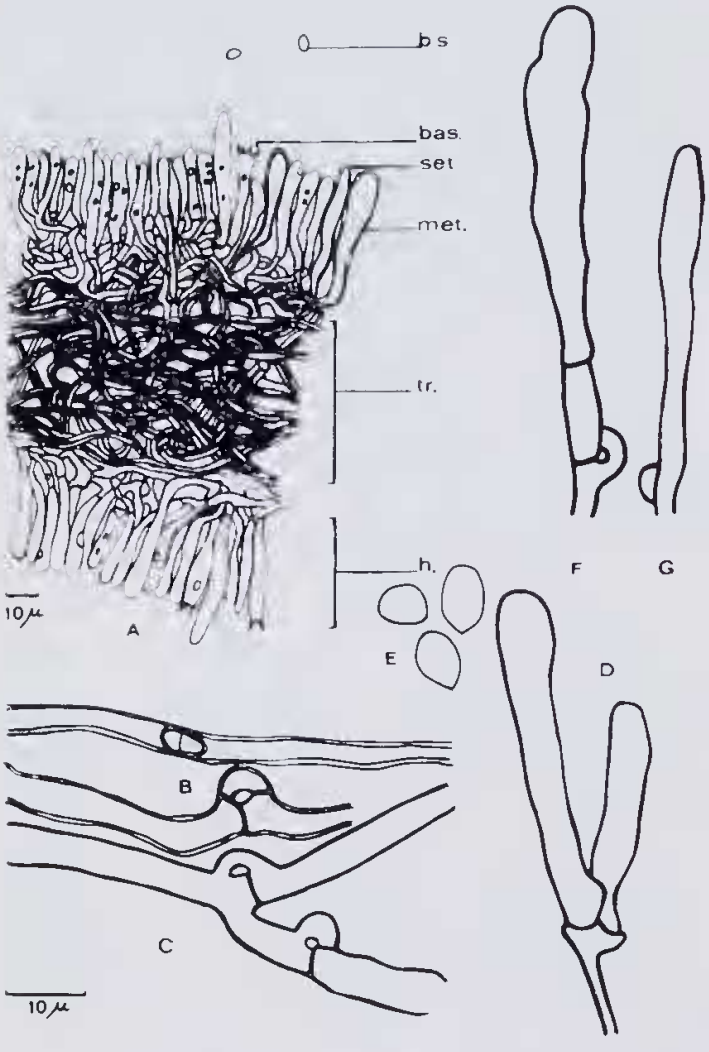


Figure 2.—*Panus fasciatus* from Western Australia. Detail from sporophore corresponding to culture number XX1. A.—Vertical section through gill showing irregular trama (tr) indistinct subhymenium, and a hymenium (h) consisting of clavate basidia (bas.), "metuloids" (met.) and "setae" (set.); basidiospores (b.s). B.—Skeletal hyphae. C.—Generative hypha. D.—Immature basidia. E.—Basidiospores. F.—Metuloid. G.—Thin-walled "seta".

2 — 4 μ, mean 2 ± 0.1 μ, and frequently branched (Fig. 2C). Trama irregular and inamyloid, subhymenium indistinct, hymenium of basidia and cystidia (Fig. 2A). Most of the basidia observed in sections were immature (Fig. 2D). Fertile basidia clavate and 20 — 36 × 4 — 7μ, mean 27 ± 0.1 × 6 ± 0.1 μ. Basidiospores hyaline, inamyloid and oblong, with smooth walls, and 4 — 7 × 3 — 5 μ, mean 5 ± 0.2 × 3 ± 0.1 μ (Fig. 2E). Cystidia originated from tramal hyphae and could be differentiated into two main types. In the first type, the cystidia were few and scattered, had thick walls and obtuse apices. They could be described as metuloids, except for the lack of crystals on their surfaces. They measured 24 — 43 × 5 — 7 μ mean 33 ± 2 × 6 ± 0.2 μ (Fig. 2F). In the second type, the cystidia were similar in size but differed in shape and wall thickness. They were thin-walled, had acute apices and were quite numerous, slightly proliferating above the hymenial surface (Fig. 2G). They resembled setae except for their thinner walls.

Cultures: macroscopic

Both isolates had indented margins consisting of appressed and submerged mycelium. The rest of the mycelial mat was raised-woolly with small aggregates of mycelium appearing near and over the inoculum after two to three weeks of growth (Figs. 3, 5). The aggregates grew larger (Figs. 4, 6), and from subsequent development were found to have been fruiting body primorida. Plates were covered after three weeks' incubation. Colour developed after four weeks: Cream Buff, then Pinkish Cinnamon, deepening to Cinnamon Buff after exposure to light. The primordia were of purplish tinge, turning to brown when exposed to light. The reverse side of the mycelial mat changed slightly to Cream Buff, particularly under the intermediate zone and inoculum. Growth rate at 25° was the same in both isolates: 2.0-2.9 cm/wk, mean ± 0.1. Reactions on tannic and gallic acid were strong with unsatisfactory growth of both isolates.

Cultures: microscopic

All hyphae examined were hyaline with thin walls or with thick refractive walls that stained poorly in phloxine. The advancing zone, aerial mycelium and submerged mycelium shared some hyphae in common. These were either thin-walled hyphae, clamped and occasionally branched (Fig. 7, a1 and a2, e1 and e2), or were wide, conspicuously clamped, with fairly thick, refractive walls characteristically branched from three clamp connections (Fig. 7, d2 and f1).

(1) *Advancing zone* (Fig. 7, a1-d2).—Two principal types of hyphae were found in the advancing zone of both isolates, XX1 and WW1. They were: (i) Long, thin-walled, hyaline hyphae with "eyelet" type of clamp connections, 4-5 μ, characteristically branched near a clamp connection and forming another clamp near the point of origin of the side branch; occasional in both isolates, (Fig 1, a1 and a2). (ii) Thin-walled, hyaline hyphae, clamped and frequently branched, branches usually short and produced in close proximity to each other, 2-4 μ wide; occasional in both isolates (Fig. 7, b1 and b2).

In addition, two more hyphal types were observed in cultures of isolate WW1. These were:— (iii) Long, thin-walled hyphae, clamped, 3-4 μ wide, with short side branches slightly naviculate in shape; rare, and arranged in a parallel fashion in the advancing zone (Fig. 7, c2). (iv) Large, thin-walled hyphae, 5-6 μ in diameter, with conspicuous clamp connections and characteristically producing branches from three clamp connections (Fig. 1, d2); rare.

(2) *Aerial mycelium* (Fig. 7, e1-j2).—The aerial mycelium in both isolates, XX1 and WW1, possessed five main types of hyphae, two of which were similar to those in the advancing zone (Fig. 7, e1, e2, f1, f2, compared with a1, a2 and d2). The other hyphal types were:— (i) Long, narrow hyphae with highly refractive walls, bearing small clamp connections and branched, either opposite to a clamp connection or near to a clamp, but more often simple branches were found (Fig. 7, g1-g2). In isolate WW1 only, this type of hypha occasionally was found to produce structures resembling chlamydospores (Fig. 7, h2), but, unlike true chlamydospores, they were not divided from the parent hypha by a septum near the base. (ii) Narrow, thick-walled hyphae, 1-2 μ wide, with lumen almost obliterated, frequently branched, resembling fibre hyphae but, unlike them, having small clamp connections, rare in XX1, occasional in WW1 (Fig. 7, i1 and i2). Clamp connections of the "eyelet" type were abundant in cultures of both isolates. Branching of the simple type was frequently found in XX1 but occasionally in WW1, where branching near a clamp connection on the parent hypha and producing another clamp near the origin of the side branch, was slightly more frequent (Fig. 7, j1 and j2). Hyphal diameter 1-5 μ mean 3 $\pm$ 0.2 μ for both isolates.

(3) *Submerged mycelium* (Fig. 7, k1-m2).—Hyphae in this area were more intensively branched than in the other areas. Three types were recognised, two of which had been found in the advancing zone and aerial mycelium (Fig. 7, k1, k2 and l1, l2). The third type of hypha was narrow, 1-3 μ wide, thin-walled and septate, with clamp connections and numerous short side branches often slightly hooked at the tips (Fig. 7, m1 and m2). The

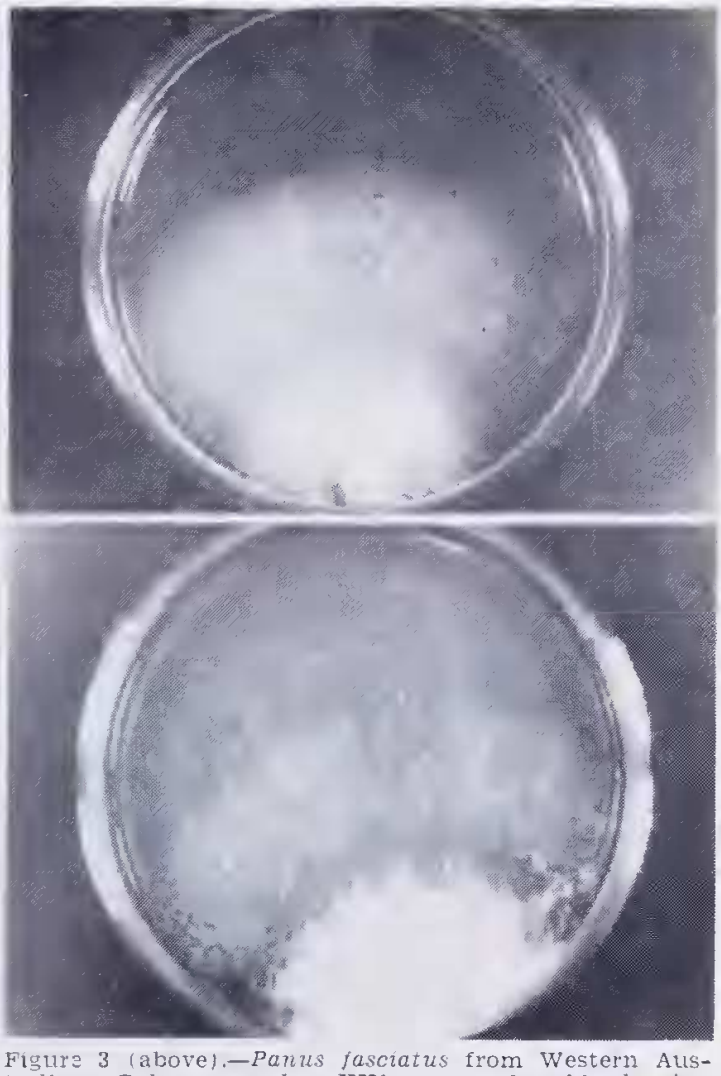


Figure 3 (above).—*Panus fasciatus* from Western Australia. Culture number XX1 two weeks old, showing uneven margin and a raised woolly texture on the mycelial mat. Mycelial mat white.

Figure 4 (below).—*Panus fasciatus* from Western Australia. Culture number XX1 four weeks old, showing that mycelium near and over the inoculum has become very dense. Fruit body primordia have developed near to the inoculum. Mycelial mat now cream buff and pinkish cinnamon.

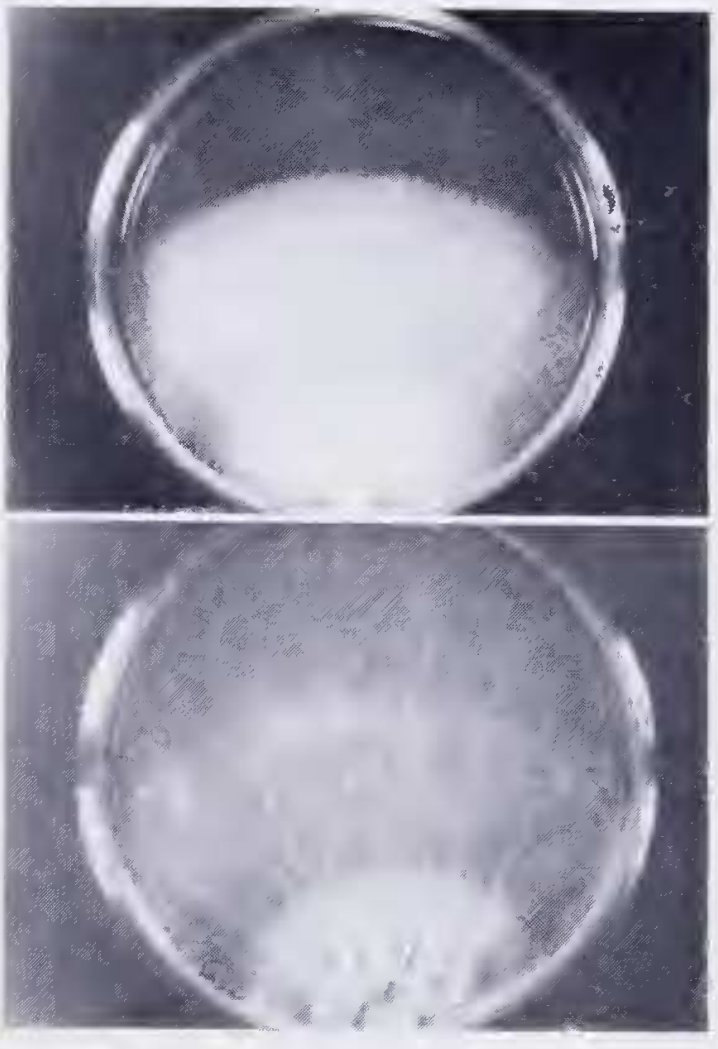


Figure 5 (above).—*Panus fasciatus* from Western Australia. Culture number WW1 two weeks old showing essentially the same features as XX1. (cf. Figure 3).

Figure 6 (below).—*Panus fasciatus* from Western Australia. Culture number WW1 after four weeks. Still showing features similar to XX1 (cf. Figure 4).

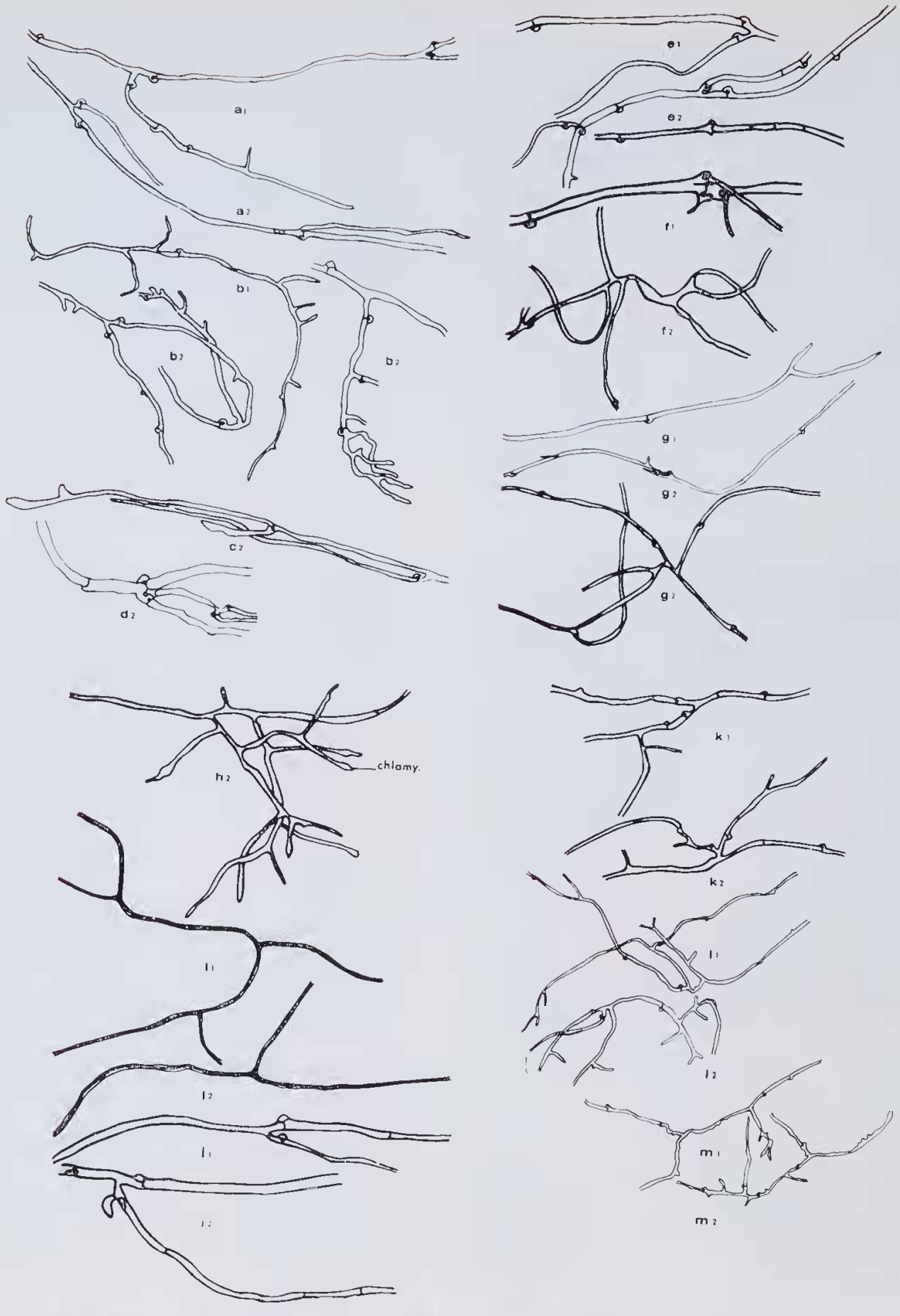


Figure 7.—*Panus fasciatus* from Western Australia. Culture numbers XX1 and WW1. Details of hyphae from advancing, aerial, and submerged mycelium. Subscript 1 refers to XX1 and subscript 2 refers to WW1.

Advancing zone, a1-d2; b1-b2, hyphae with branches produced in close proximity; c2, hyphae with short side branches slightly naviculate in shape; d2, wide hyphae with conspicuous clamp connections characteristically branched at three clamp connections. Aerial Mycelium, e1-j2; f2, hyphae irregularly enlarged; g1-g2, hyphae with highly refractive walls; h2, hyphae with terminal swellings resembling chlamydospores (chlamy.) except for the absence of a septum; i2, thick-walled "fibre hyphae". Submerged mycelium, k1-m2; m1-m2, hyphae with short lateral branches straight or slightly hooked at the tips.

Table 1

Comparison of sporophore microstructure of Panus fasciatus *and* Lentinus terrestris

	P. fasciatus (W.A.)		*P. fasciatus* (N.S.W.)		*L. terrestris* (N.S.W.)	
	Range	Mean	Range	Mean	Range	Mean
Basidia	20–36 × 4–7	27 ± 0·1 × 6 ± 0·1	22–54 × 4–7	31 ± 1·8 × 6 ± 0·2	18–40 × 4–9	29 ± 1·2 × 6 ± 0·3
Basidiospores	4–7 × 3–5	5 ± 0·2 × 3 ± 0·1	4–7 × 3–5	6 ± 0·1 × 4 ± 0·1	5–9 × 4–5	6 + 0·5 × 4 ± 0·2
Skeletal hyphae	3–5	3 ± 0·1	2–5	2 ± 0·2	3–5	4 ± 0·2
Generative hyphae	2–4	2 ± 0·1	2–4	3 ± 0·1	2–4	3 ± 0·1
Metuloids	24–43 × 5–7	33 ± 2 · 6 ± 0·2	22–36 × 4–7	31 ± 0·7	Nil	Nil

All measurements in μ.

"eyelet" type of clamp connection was abundant in the submerged mycelium of both isolates. Hyphal diameter 1-6 μ, mean 3 $\pm$ 0.2 μ for both isolates.

Comparison of Panus fasciatus from Western Australia and New South Wales

Specimens of *Panus fasciatus* from Nambucca Heads, New South Wales, (DFP 5365) showed strong resemblances to those from Western Australia in the macro- and micro-features of the sporophores. They both had brown, densely hispid, deeply infundibuliform pilei; decurrent gills with entire edges; brown, hispid stipes (Fig. 1 and 8). Microscopically they were similar in having a white context, filamentous cuticle, and an irregular, inamyloid trama consisting of skeletal and generative hyphae. The subhymenium was indistinct in both specimens and the hymenium consisted of essentially the same elements. These were clavate basidia; oblong, hyaline, smooth, basidiospores; metuloids and setae. There was a slight difference in size of these elements between the two specimens (Table 1), and the setae from the New South Wales specimen had thicker walls (Fig. 9, f). Cultures from New South Wales did show differences in texture, colour, odour and growth rate from the Western Australian isolates, although reactions on gallic and tannic acid media

Figure 8.—*Panus fasciatus* from New South Wales. Sporophore corresponding to culture number DFP 5365. Note growth from a pseudosclerotium.

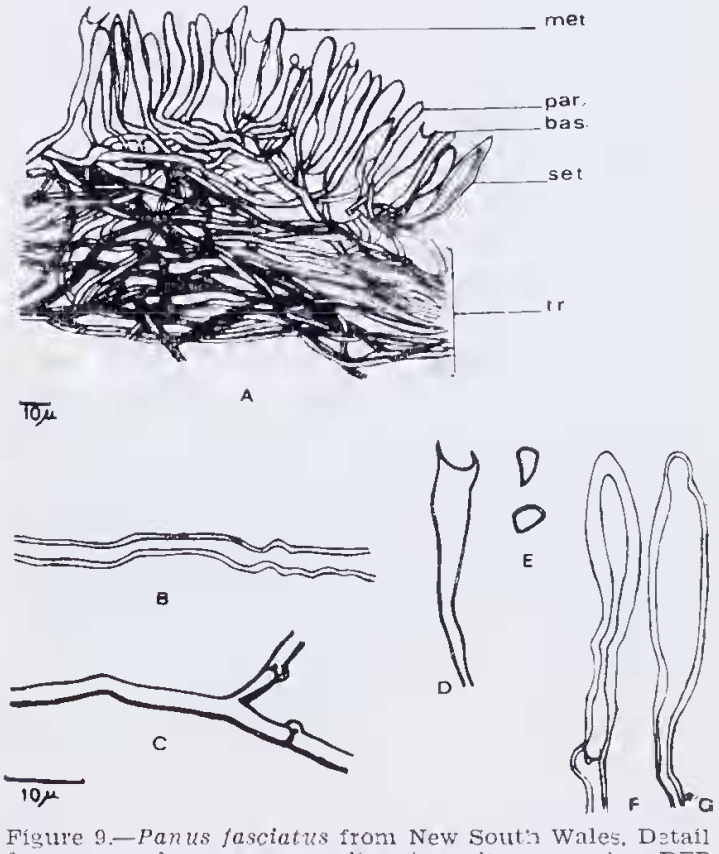
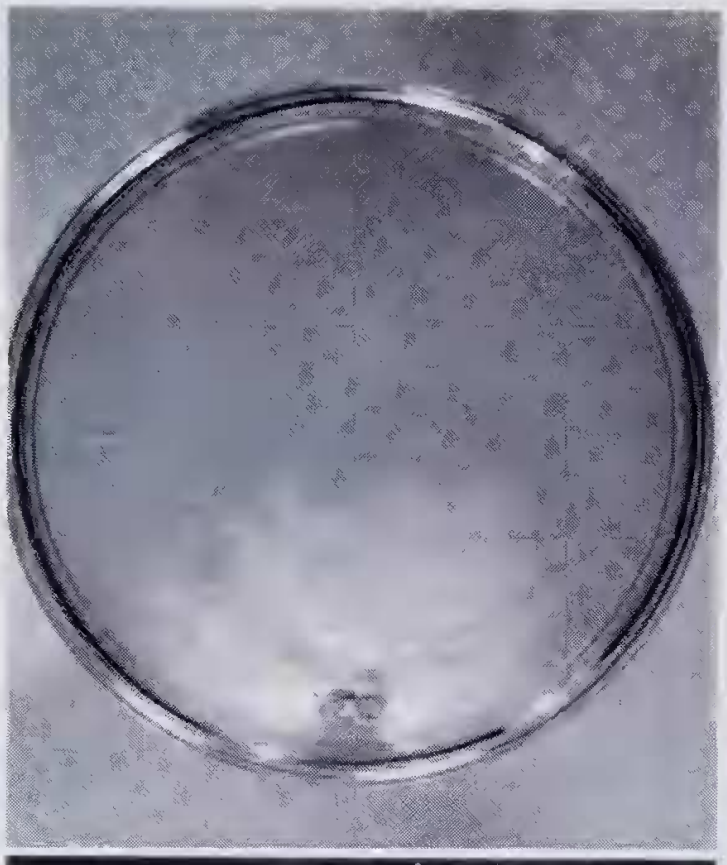


Figure 9.—*Panus fasciatus* from New South Wales. Detail from sporophore corresponding to culture number DFP 5365. A.—Vertical section through gill, showing irregular trama (tr.), basidia (bas.) paraphysate hyphae (par.) metuloids (met.) and setae (set.). B.—Skeletal hypha. C.—Generative hypha. D.—Basidium. E.—Basidiospores. F.—"Seta". G.—"Metuloid".



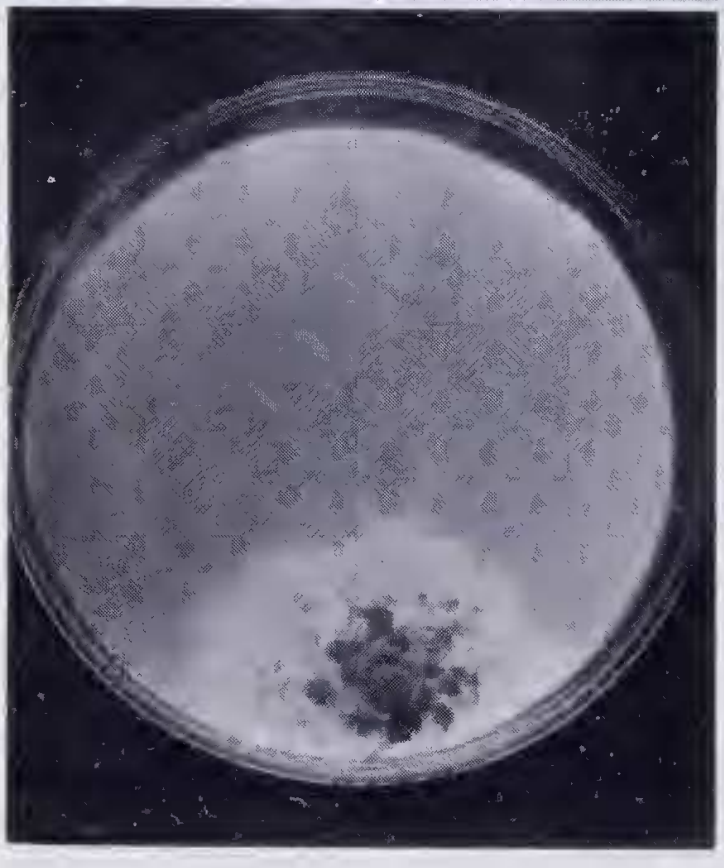


Figure 10 (above).—*Panus fasciatus* from New South Wales. Culture DFP 5365 two weeks old with a raised, silky texture in the younger parts and a sub-felty texture in the older parts of the mycelial mat, which was maize yellow or cream-buff in colour.

Figure 11 (below).—*Panus fasciatus* from New South Wales. Culture DFP 5365 after four weeks, showing little change except for the development of small compact lumps of mycelium over the inoculum.

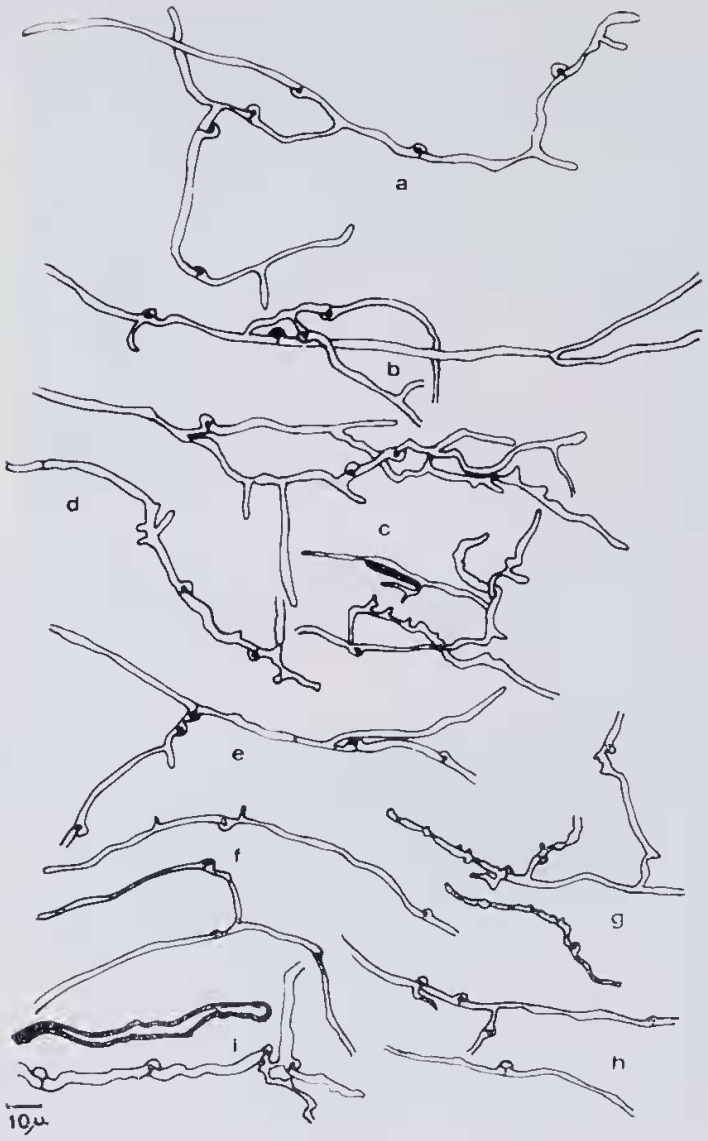


Figure 12.—*Panus fasciatus* from New South Wales. Culture number DFP 5365. a-c, hyphae from the advancing zone; d-f, from the aerial mycelium; g-i, from the submerged mycelium.

were similar. In spite of the differences in the macroscopic appearance of the cultures (Figs. 10 and 11), two hyphal structures were found to be identical between the two isolates (Fig. 12, a, was similar to Fig. 7, a1 and a2, while Fig. 12, c and g resembled Fig. 7, m1 and m2). It can be concluded that the *Panus fasciatus* from New South Wales was the same species as that from Western Australia, but a different variety.

Comparison of Lentinus terrestris with Panus fasciatus from Western Australia

Cleland (1934 p. 171) suggested *Lentinus terrestris* Lloyd (1925) as a probable synonym of *Panus fasciatus* (quoted by him as *L. fasciatus* Fr.). Because of Cleland's suggestion, supported by co-types in his possession, named specimens of *L. terrestris* were obtained from the Division of Forest Products, C.S.I.R.O., Melbourne for comparison with specimens of *Panus fasciatus* from Western Australia. The collection supplied was DFP 7396 collected on Mount Banda Banda, Wauchope, N.S.W., September, 1959.

Lentinus terrestris showed differences from *Panus fasciatus* in the macro-and micro-features of the sporophores and in the macro-

Figure 13.—*Lentinus terrestris* Lloyd. Sporophores corresponding to culture number DFP 7396.

scopic and microscopic appearance of the cultures.

Morphologically, *L. terrestris* differed from *P. fasciatus* in having pilei that were slightly depressed at the centres, gills that were dentate instead of entire, and large sporophores that were also hispid but with shorter abhymenial hairs. *L. terrestris* grew from a pseudosclerotium in soil. (Fig. 1 and Fig. 13).

Microscopically (Fig. 14) both sporophores appeared similar but unlike *P. fasciatus*, the trama in *L. terrestris* was subregular (Fig. 14, A), although it was also composed of inamyloid, thick-walled skeletal hyphae. Generative and skeletal hyphae appeared similar in both species and were of similar size (Table 1). The subhymenium was indistinct and the hymenium was composed of essentially the same elements in both species. These were clavate-shaped basidia; hyaline, inamyloid, smooth basidiospores, and setae. However, unlike *P. fasciatus*, *L. terrestris* had no metuloids. Basidia and basidiospores were larger in *L. terrestris* (Table 1) and the setae in *L. terrestris* had uniformally thick walls and were not thin-walled as in *P. fasciatus* from Western Australia.

Cultures of *L. terrestris* (Fig. 15 and 16) differed in texture, colour and growth rate from cultures of *P. fasciatus*. *L. terrestris* had a cottony mycelial mat which became woolly during later periods of incubation. *P. fasciatus* had a woolly texture throughout the whole period of incubation, with the mycelium becoming slightly appressed as the cultures grew older. Growth rate in *L. terrestris* was slower. The mycelial mat was Pale Pinkish Buff, Pinkish Buff or

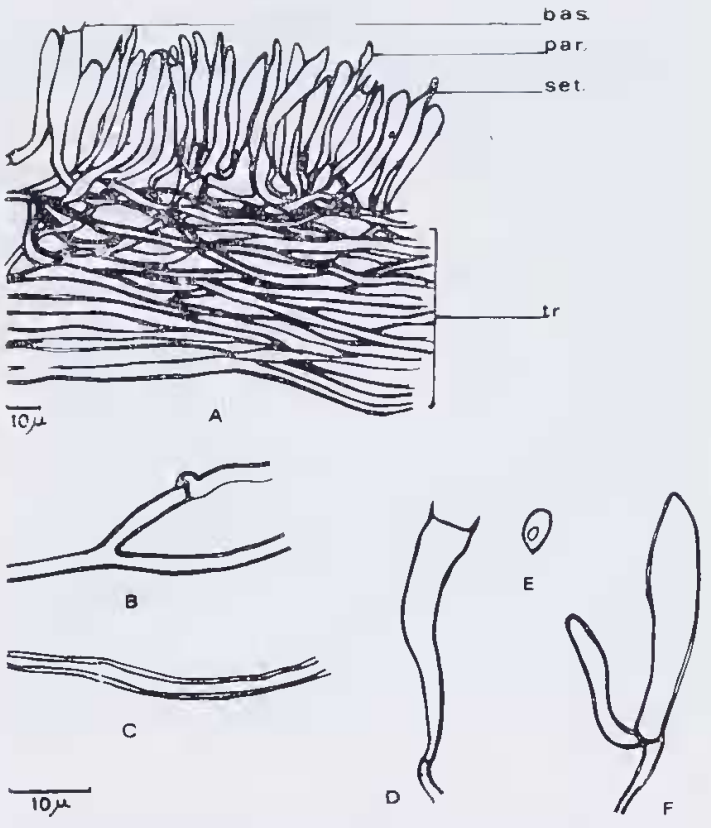


Figure 14.—*Lentinus terrestris* Lloyd. Sporophore corresponding to culture number DFP 7396. A.—Vertical section through gill showed subregular trama (tr.) indistinct subhymenium and hymenium consisting of clavate-shaped basidia (bas.) setae (set.) and paraphysate hyphae (par.) Note absence of metuloids. B.—Generative hypha, thin-walled, clamped and branched similar to those of *P. fasciatus*. C.—Skeletal hypha, thick-walled and rarely branched, resembling those of *P. fasciatus*. D.—Clavate basidium, larger than *P. fasciatus*. E.—*Basidiospores* similar to those of *P. fasciatus* except for the larger size. F.—Seta, thick-walled.

Light Ochraceous Salmon in *L. terrestris* whereas it was Cream Buff or Pinkish Cinnamon in *P. fasciatus*. Reactions on tannic and gallic acid media differed from *P. fasciatus* only in that on gallic acid being weak.

Microscopically, the hyphae in cultures of *L. terrestris* differed from *P. fasciatus* in the absence of clamp connections (Fig. 17 compared with Fig. 7), the presence of dendritic hyphae (Fig. 17, c) and in having true chlamydospores in the aerial and submerged mycelium in *L. terrestris* (Fig. 17, 1, compare with Fig. 7, h2).

The general characters of the *L. terrestris* isolate, particularly the inamyloid spores and toothed gills, are consistent with its being retained in the genus *Lentinus*, differing from *P. fasciatus* even at this, the generic, level.

Acknowledgements

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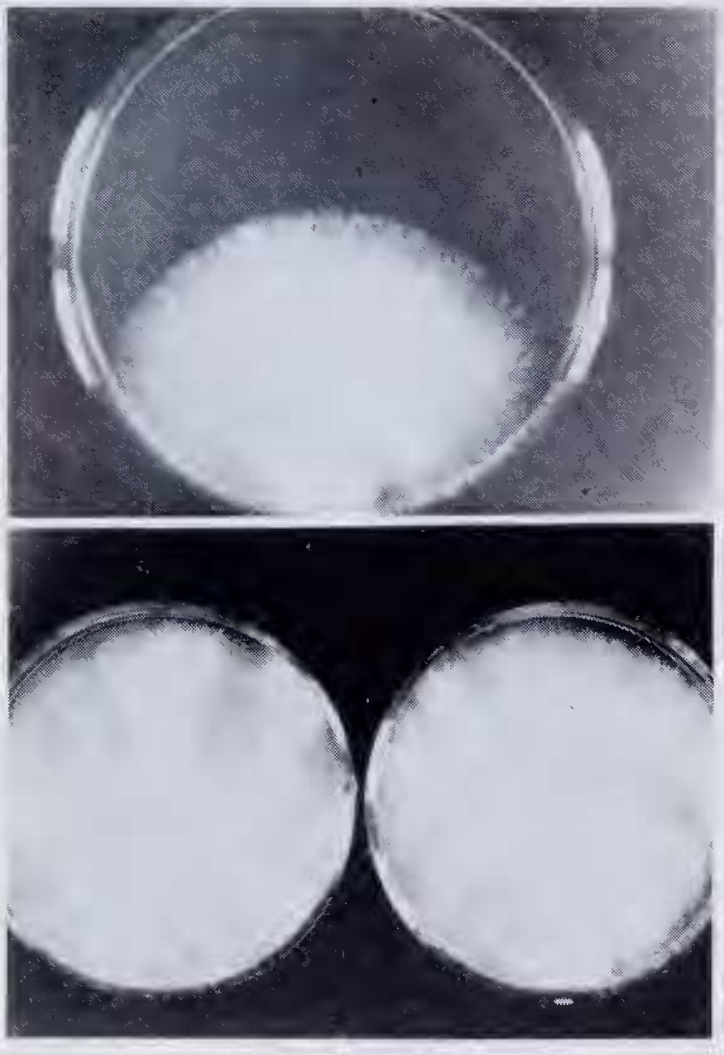

Figure 15 (above).—*Lentinus terrestris* Lloyd. Culture DFP 7396 two weeks old, showing white aerial mycelium, uneven margin and raised cottony-woolly texture.

Figure 16 (below).—*Lentinus terrestris* Lloyd. Culture DFP 7396 four weeks old, showing zones and radial striations on the mycelial mat. Colour developed over the inoculum, but no fruiting bodies even after exposure to light.

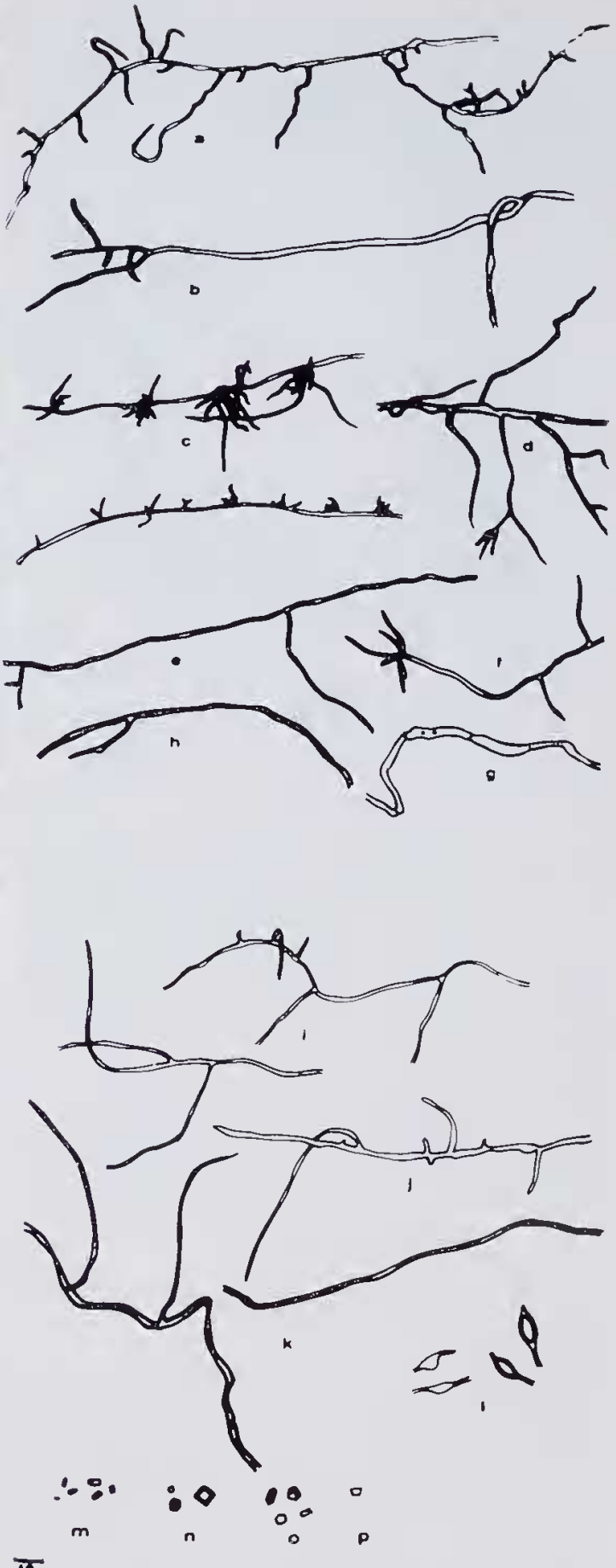

Figure 17.—*Lentinus terrestris* Lloyd. Culture number DFP 7396. a-d, hyphae from the advancing zone; e-h, hyphae from the aerial mycelium; i-k, hyphae from the submerged mycelium; a, hyphae intensively branched; c and f, dendritic hyphae, observed in *L. terrestris* only; d, hyphae with highly refractive walls, and numerous short side branches; m-p, crystals.

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